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APPLICATION OF DIFFERENTIAL EQUATIONS TO THE STUDY OF THE THYROID SYSTEM

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1. Introduction

This paper deals with the application of newly developed techniques of data analysis to studies presently in progress on the thyroid system using isotope tracers. In general, the biological system that one encounters in an in vivo tracer experiment is quite complex. The tracer experiment alone is inadequate to define it, and one tries to construct a substitute system from the data that will approximate the behavior of the actual system. This substitute system serves as a model for the true system and its complexity or resolution is no greater than the experimental data justify. A degree of arbitrariness is present in deciding how complex a model should be, and this depends on statistical, physiological, and biochemical considerations.

When a model compatible with all the available information on the system is derived, one tries to correlate the parameters of the model with known physiological and biochemical entities. One also tries to correlate changes in the values of the model parameters with known clinical or physiological abnormalities. Successes or failures of these attempts serve as additional criteria in the development of an acceptable model.

The reasons for choosing to discuss the thyroid system are: first, the thyroid system is sufficiently complex to represent many of the problems one encounters in the analysis of tracer experiments in general; second, we have a number of studies on this system that are presently being analyzed. In collaboration with Drs. D. Becker of the New York Hospital, and M. Sonenberg and R. S. Benua of the Sloan Kettering Institute, New York, kinetic studies were performed on a number of patients with various thyroid abnormalities. Ezra Shahn of the Institute for Arthritis and Metabolic Diseases has participated in the development of some of the techniques described and programmed the computational procedures for an IBM 704 computer.

2. The "thyroid system"

The term "thyroid system" is defined to include the major phases involved in the production, utilization, and regulation of thyroid hormone. The hormone is

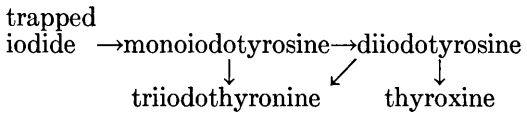
synthesized in the thyroid gland from components supplied to it by the blood. At least two forms of hormone are known to exist: thyroxine (T_4) and triiodothyronine (T_3). The hormones are released from the thyroid to the circulation and are distributed widely in the extracellular and intracellular spaces. They play a role in the metabolism of cells in as yet an unknown fashion and eventually become degraded.

The rate of synthesis and release of the thyroid hormone by the thyroid is controlled by another hormone known as Thyroid Stimulating Hormone (TSH). This hormone is produced by the pituitary and its rate of secretion is controlled by the level of thyroid hormone in the circulation. Hence, in a normal individual, the level of thyroid hormone is regulated through the TSH feedback loop.

3. Iodine pathways

Iodine is one of the elements that constitute the hormones T_3 and T_4, and one can trace some of the pathways of the hormone by using radioiodine as a label.

When iodide enters the circulation it distributes rapidly in the vascular, extravascular-extracellular, and to a limited extent in the intracellular spaces. The kidneys filter some of the circulating iodide and excrete it into the urine. The thyroid traps the remaining iodide and concentrates it to levels much higher than in the circulating plasma. The trapped iodide may either be released back to the circulating plasma or be bound into the thyroid hormones. The biochemical steps for the synthesis of the hormones are not fully known, but the following stages are believed [1] to be involved:



Thyroxine and triiodothyronine are released from the thyroid to the circulation where they are bound to proteins and become distributed in the vascular, extracellular and intracellular spaces. The iodine bound to proteins may be isolated chemically and is known as Protein Bound Iodine (PBI). When the hormones are degraded, the iodine is released in inorganic form and is free to repeat the entire cycle again.

The lengths of times for the different processes range from minutes to months. The initial rate of distribution of iodide is of the order of minutes and its rate of loss from the circulation is of the order of hours. On the other hand, the rate of secretion of T_4 from the thyroid is about 1 to 2 per cent per day and its rate of degradation in the circulation is about 10 per cent per day.

4. Compartmental model for radioiodine

If each state of iodine discussed above is defined as a compartment, then the entire cycle of iodine metabolism may be represented schematically as shown in

figure 1. The circles represent the iodine compartments and the arrows are paths of transition.

The TSH regulatory phases are not shown in figure 1 because they do not

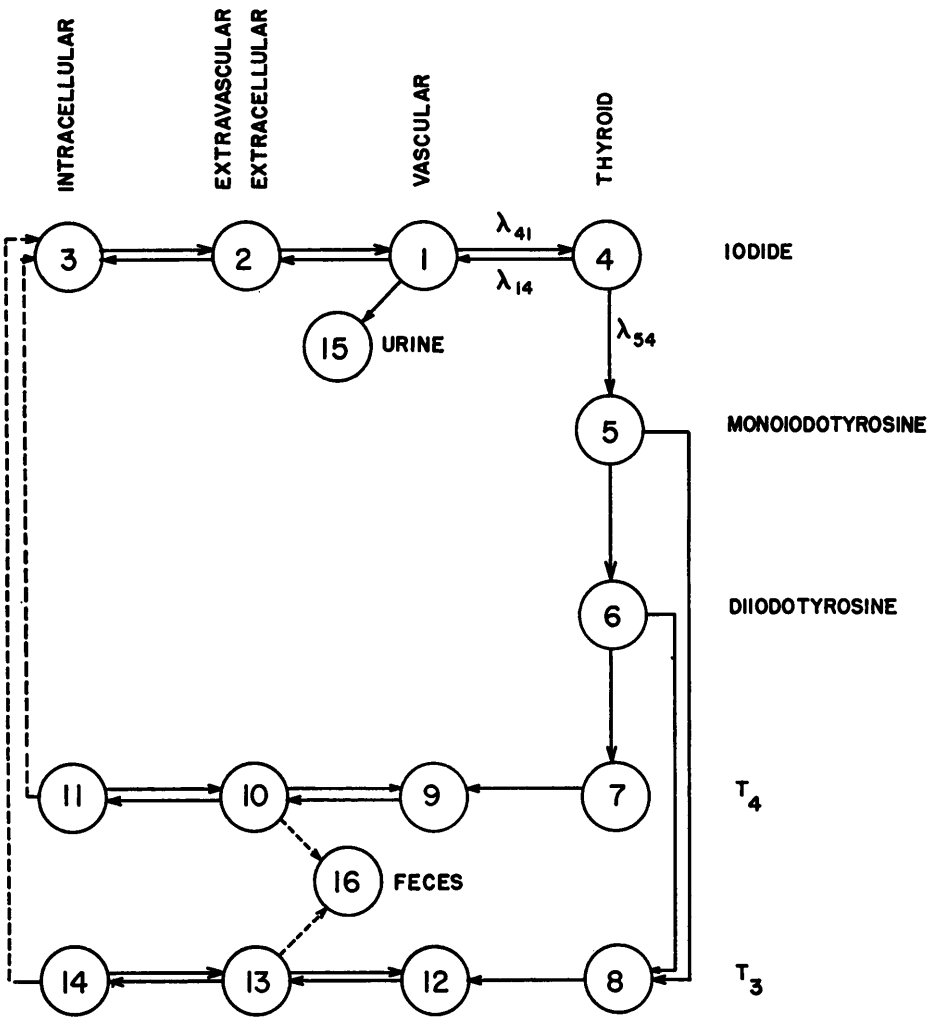


FIGURE 1

Schematic representation of the major known stages
in the metabolism of iodine.
(Dotted lines indicate uncertainty in their point of origin.)
(Vertical and horizontal titles refer to entire column and row, respectively.)

contain iodine. It is known that TSH affects the rate of iodine uptake by the thyroid and the rate of the hormone secretion from the thyroid, but its exact sites of action are not known.

The probabilities of transition per unit time from one compartment to another

are usually independent of each other, but depend in a nonlinear fashion on many variables such as diffusion constants, chemical reactions, concentration of enzymes, hormones, etc. When the concentrations of the various components in the system remain constant during the course of the experiment and the transition probabilities between compartments do not change, the kinetics of a tracer isotope are linear. If it is further assumed that mixing within each compartment is rapid, the kinetics of a compartmental model may be represented by a set of differential equations:

$$\dot{q}_i = -\lambda_{ii}q_i + \sum_{j \neq i}^{n} \lambda_{ij}q_j, \qquad i = 1, 2, \cdots, n, \tag{1}$$

where q_i is the amount of labeled material in the ith compartment and λ_{ij} is the probability of transition per unit time for a labeled atom from the jth to the ith compartment. λ_{ii} is defined as the sum of all probabilities that an atom will leave compartment i; n is the number of compartments assumed for the system. (A comprehensive bibliography on linear compartmental models appears in [2].)

The compartmentalization is, of course, an invention for purposes of analysis, since the physiological states do contain finer structure. The only justification for the compartmental analysis is that in many cases it is a good first model for approximating the system and that it yields sometimes useful parameters in elucidating mechanisms of action.

The solution of the set of differential equations (1) is [3]

$$q_i = \sum_{j=1}^{n} A_{ij}\, e^{-\alpha_j t}, \qquad i = 1, 2, \cdots, n \tag{2}$$

where the α_j are eigenvalues of the λ_{ij} matrix and the A_{ij} are functions of the λ_{ij} and the initial conditions of the experiment.

In practice the collected data on a system usually include the initial conditions of the experiment and measures for the amount of isotope in certain compartments at various times. The system configuration is unknown, and in order to learn about it, one needs to determine the values of the λ_{ij} or some linear combinations of them. Theoretically, if all the A_{ij} and α_j are known, the following matrix relation [3] permits the calculation of the λ_{ij}.

$$|\lambda| = |A|\,|\alpha|\,|A^{-1}|, \tag{3}$$

where α is a diagonal matrix of the α_j elements. Certain difficulties, however, prevent the use of this expression directly. A typical radioiodine tracer experiment in a human will be discussed to bring this out. Reference will be made to figure 1.

5. Tracer study

A known amount of radioiodide is injected into the circulation (compartment 1) and measurements of radioactivity are obtained at about 10 minutes, 1, 2, 4, 8, 24 hours, and 2, 3, 4, 6, 8, 10, 12, 14 days for the following:

(a) Blood samples are collected and chemical separation of iodide and PBI in

the plasma is made. Radioactivity in each fraction is determined. Sometimes breakdown of PBI into T_4 and T_3 is also made. The measurements yield values for the amount of labeled iodide and PBI per unit volume of plasma. During the first day most of the activity in the blood is iodide and the values obtained for PBI are relatively inaccurate. Similarly, after the first day most of the activity in the plasma is due to PBI and the accuracy of the iodide values is quite poor.

(b) Total radioactivity in the thyroid is measured with a radiation detector. This determination may have a systematic error of about ± 20 per cent due to the techniques involved. No differentiation between the different compartments in the thyroid is possible. In addition, about 5 to 10 per cent of the total radioactivity in the body is also "seen" and measured by the detector.

(c) Urine samples are collected and the radioactivity for each collection is measured. The collections of urine are not always complete because some patients lose specimens.

On some patients separate studies are made with labeled thyroxine or triiodothyronine instead of iodide. Measurements similar to those for the iodide studies are then also made.

In terms of the model in figure 1 the measurements correspond to the following quantities:

$$\begin{aligned}
\text{Plasma iodide} &= k_1 q_1 \\
\text{Plasma PBI} &= k_9 q_9 + k_{12} q_{12} \\
\text{Thyroid} &= k(q_4 + q_5 + q_6 + q_7 + q_8) + k_2 q_1 + k_3 q_9 + k_4 q_{12} \\
&\quad + k_5 q_2 + k_6 q_3 + k_7 q_{10} + k_8 q_{11} + k_{10} q_{13} + k_{11} q_{14} \\
\text{Urine} &= \int_{t_1}^{t_2} dq_{15}
\end{aligned}$$

The k_i are usually unknown proportionality constants.

It is obvious from the measurements that use of equation (3) directly is impossible. The difficulties may be restated as follows:

1. The configuration of the system studied is not necessarily known a priori.
2. Not all compartments are accessible for measurements.
3. Not all times are equally accessible for measurements.
4. Some measurements represent a linear combination of compartments.
5. The collected data have fluctuations due to biological variations and experimental errors.
6. The data are not sufficient to resolve the system fully.

The object of the analysis is to derive a model that will approximate the true system and be consistent with all the experimental data collected and with any other information available on the system.

6. Model for radioiodine kinetics

Since the system cannot be solved directly and a model is desired, one can start with as simple a model as seems reasonable.

It was already pointed out that the rates of iodide distribution in compartments 2 and 3 are very rapid, and no detailed measurements over this time interval were made. Consequently, one cannot expect to resolve the three iodide compartments and the iodide space will be approximated by a single compartment.

Although the measurement over the thyroid includes several compartments, it is doubtful that the data will permit their resolution. The thyroid will therefore be represented by a single compartment.

The PBI measurements, especially after the first day, are mostly T_4 with probably less than 10 per cent of T_3 and iodide. Hence a single compartment will be used initially to represent PBI measurements.

The model for the simplified radioiodine system is shown in figure 2. The choice of this model may seem reasonable, but obviously is still arbitrary. The adequacy of the model will depend on how well it fits the experimental data.

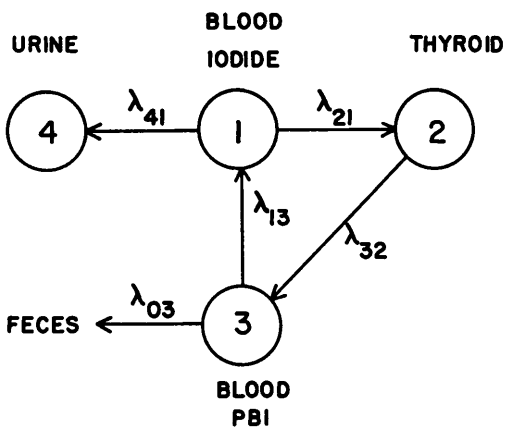


FIGURE 2

Initial model for radioiodine kinetics.

In terms of the model of figure 2, the measured quantities can be represented as follows:

Plasma iodide $= k_1 q_1$

Plasma PBI $= k_3 q_3$

Thyroid $= k_2 q_2 + k_4 q_1 + k_5 q_3$

Urine $= \int_{t_1}^{t_2} dq_4$

A "best" fit for the model may be obtained by deriving a set of values for the parameters λ_{ij} and the unknown constants k_i that will result in a least sum of squares of the deviations between the experimental and calculated values for the data. The acceptance of the model will depend on how good the "best" fit is.

A method for obtaining a "least sum of squares" set of values for the parameters of the model and a criterion for judging whether the least sum of squares solution is acceptable will now be presented. Further discussion on the radioiodine model will follow.

7. Least squares solution for model parameters

Given the initial conditions of the experiment and a set of initial estimates for the λ_{ij} and k_i, a value corresponding to each measured quantity may be calculated. Assuming that the values for a least squares fit equal those of the initial estimate plus a first order correction term, we may write [4] for each datum, l, that its value $F_l(\lambda_{ij}, k_h)$ for a least squares fit is

$$(4) \qquad F_l(\lambda_{ij}, k_h) = F_l(\lambda_{ij}^0, k_h^0) + \sum_{i,j=1}^{n} \frac{\partial F_l(\lambda_{ij}^0, k_h^0)}{\partial \lambda_{ij}} \delta\lambda_{ij} + \sum_{h=1}^{n} \frac{\partial F_l(\lambda_{ij}^0, k_h^0)}{\partial k_h} \delta k_h, \qquad l = 1, 2, \cdots, m,$$

where the superscript ^{0} refers to the initial estimate values, and m is the number of data points.

Equations (4) form a set of equations of condition in the variables $\delta\lambda_{ij}$ and δk_i from which a set of normal equations may be set up and corrections $\delta\lambda_{ij}$ and δk_i and their variance covariance matrix calculated. Since, in general, first order correction terms are inadequate, the corrected values of the λ_{ij} and k_i may be used as new initial estimates and the entire procedure repeated.

Unfortunately this procedure does not guarantee convergence. It works well if a unique minimum in the sum of squares surface exists and if the initial estimates are close enough to the least squares values so that first order corrections are nearly adequate. Either one or both of these conditions are quite frequently violated in practice.

It is easy to show why a unique minimum in the sum of squares surface may not exist. Equation (3) shows that solution for each λ_{ij} may be obtained if all the A_{ij} and α_j are known. This means that for any system studied every compartment must be measured with sufficient frequency or accuracy to permit a unique determination of all A_{ij} and α_j. It is seldom that such complete data can be obtained on a biological system and therefore the unique solution of all λ_{ij} becomes impossible.

A unique solution may be obtained with incomplete data only if certain constraints are imposed on the model. The constraints may be applied as a result of independent information about the model, or may be postulated merely to permit a solution. For example, certain λ_{ij} were set equal to zero in figures 1 and 2 on the basis of studies reported in the literature.

Corresponding to each constraint imposed on the model a dependence is established between the parameters of the fit, the A_{ij} and α_i, and as a result less data is necessary to solve for the model parameters. The relations between the A_{ij} and α_j produced by one or more constraints may become quite complex

and usually it is not at all obvious a priori whether the available data are adequate to solve for the model parameters uniquely.

Nonuniqueness may be detected in the numerical calculations. It results in a set of ill-conditioned normal equations, unusually large and distorted corrections, and divergence in the sum of squares. This may be eliminated by adding more constraints to the model.

If the initial estimates of the values of the parameters are significantly different from the best values, it is conceivable that the calculations may lead to a local minimum in the sum of squares surface that is different from the absolute minimum. If the value for the local minimum is nearly the same as that for the absolute minimum, the solution is perfectly acceptable, since it is one of at least two or possibly many solutions. There is no way, of course, to know about the presence of the other solutions unless one starts with many different initial estimates and hopes to converge eventually to all existing minima.

To aid in judging whether the sum of squares obtained differs significantly from the least sum of squares, a reference sum of squares is introduced as a reasonable estimate of a lower limit for the least sum of squares.

The least sum of squares in the solution for a model must always be equal to or greater than the least sum of squares in the solution of the same model with one degree of freedom added. More specifically, a general n compartment model has a solution with a least sum of squares that is equal to or less than that for any constrained n compartment model, or any other compartmental model of order less than n.

Thus, for every special model considered, a least squares solution of a more general model is also obtained as a reference. When the sum of squares for the two solutions are nearly the same, the solution for the special model is accepted. When the sum of squares for the special model is significantly greater than that for the reference model, the solution of the special model is questioned.

It is assumed here that a solution obtained for any model that is not unique is not likely to have a sum of squares much different from the least sum of squares. The reference model will probably be nonunique because of the added degrees of freedom.

It is important that as few degrees of freedom as possible be added to the special model to obtain a reference model, and that the number of compartments not be increased, if possible. The reason for this is that theoretically, given enough compartments, a perfect fit of the data can be obtained. This, of course, would make the reference model meaningless.

If the sum of squares for the solution of the special model is significantly higher than that for the reference model, two interpretations are possible. One is that the solution obtained is not a least squares solution. The second is that the special model is inconsistent with the experimental data.

The second possibility cannot be accepted until the first one is eliminated. This is no trivial problem. One can start with many different initial estimates for

the special model, obtain other solutions and compare them. This, however, is very time consuming and does not guarantee that the least squares solution may not be missed. Other procedures have also been considered, but hold little promise so far for simplicity and universal applicability.

If the solution of the special model does differ significantly from the reference model in its sum of squares, and one has confidence from experience and insight that the solution is not near the true minimum, then the interpretation must be that the special model under consideration is inconsistent with the data and should be modified.

Although the theoretical aspects of the problems presented here are not fully resolved, a number of empirical "tricks" have been incorporated in the calculations to permit practical applications of the procedures outlined. Further details of this will be discussed elsewhere [5].

8. Computer program

The calculations of the model parameter values for a least squares fit of the data are performed on an IBM 704 digital computer. The major features of the program are

1. A general model with up to 15 compartments can be treated.
2. Any λ_{ij} and k_i may be either fixed or variable. When variable, its range of variation may be specified.
3. The original experimental data may be fed into the computer.
4. A statistical weight may be assigned to each datum.
5. The initial condition of the experiment may be entered directly.
6. Initial estimates for all λ_{ij} and k_i are necessary.

After all the information is read in, the program calculates theoretical values corresponding to the experimental data. The partial derivative of each calculated value with respect to each variable parameter of the model is also calculated. A set of equations of condition, as indicated by equation (4), are formed from the partial derivatives. The normal equations are computed from the equations of condition, and a correction vector for the variable parameters is obtained.

The correction vector is tentatively accepted and new theoretical values corresponding to the data are calculated. The deviations between the theoretical and experimental values are obtained and compared to similar deviations before the correction was made. From this comparison an adjustment in the magnitude of the correction vector is made to give the best possible improvement in the sum of squares. The "best" correction vector so obtained is then used for the final adjustment of the values of the model parameters. These then serve as initial estimates for the next iteration. The use of this procedure for the modification of the originally calculated correction vector prevents the acceptance of any set of corrections that could cause divergence in the sum of squares, and greatly accelerates the rate of convergence of the entire procedure.

When the sum of squares for an iteration is within a specified fraction of that for the previous iteration, the procedure is terminated.

More details on the program will be reported separately [5].

9. Formulation of radioiodine model

The described techniques have been applied to data obtained on a number of patients with various thyroid abnormalities. Much of the data for the different patients varied both in the compartments sampled and in the accuracy of the measurements. The point of view followed in deriving a compatible model was that qualitatively the same model must apply to all studies (or patients) and that differences between studies (or patients) are quantitative only. Thus, when a model is proposed for one kinetic study, the same model is also used for the other studies. If the model does not seem to fit some studies, it is modified and tested again on all the studies.

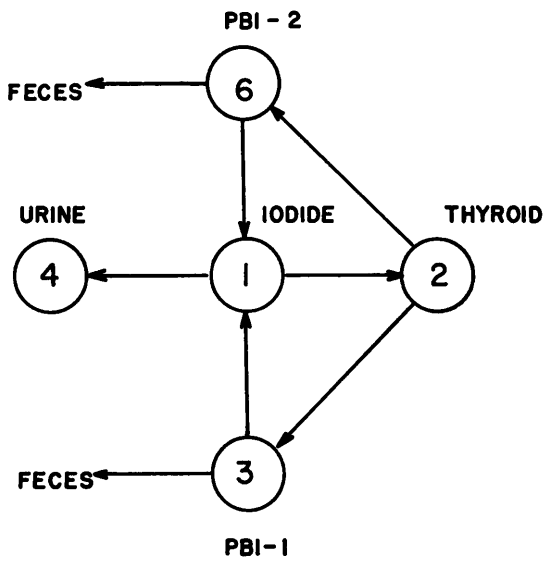


FIGURE 3

Radioiodine model with 2 PBI compartments.

The first model proposed for the radioiodine system was that discussed earlier and shown in figure 2. It was soon found to be inconsistent with a number of studies. The systematic deviations that were obtained between the experimental and theoretical values suggested that the PBI data represent a sum of two compartments. This is consistent with the known existence of T_4 and T_3. The modified model is shown in figure 3 and a good fit of the data was obtained for it. In several studies, however, the calculated size of the PBI space was considerably different from the value suggested in the literature. When the value for the PBI

space was fixed to agree with that in the literature, a consistent fit could not be obtained.

A second thyroid compartment was introduced as the next modification, and the new model is shown in figure 4. This model fitted the data quite well, except for those studies in which daily urine radioactivities over a 2-week period were measured. In these studies the measured values of the daily urines consistently deviated from the calculated ones, and this could only be eliminated by shifting the inconsistency elsewhere in the fit. Further evidence for the inconsistency of the model came from a study on one patient using labeled T_4 as a tracer. The size of the T_4 space and its turnover rate could readily be determined from this

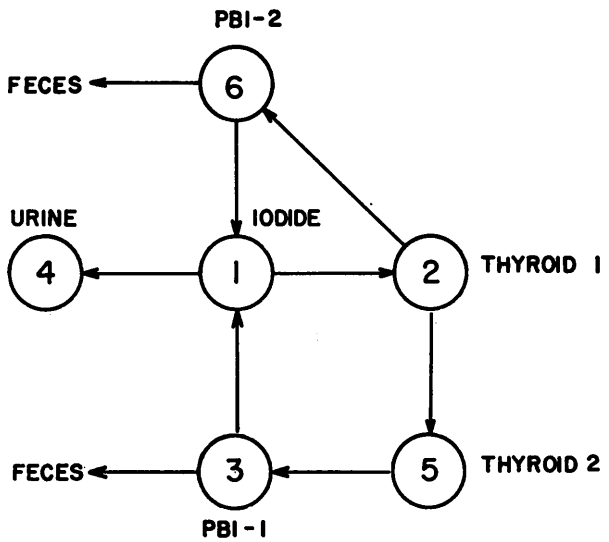


FIGURE 4

Radioiodine model with 2 thyroid compartments.

study alone. Inserting these values in the study on the same patient using an iodide tracer, one could not obtain a good fit of the data.

A path was then introduced for the direct release of iodide by the thyroid. (This path is in addition to the iodide released from the thyroid trap.) The possibility for such a path has been suggested in the literature before, but little evidence for its presence has been presented.

Introduction of the path from compartment 5, figure 5, did not remove the inconsistency of the fit. A feedback path from compartment 2, figure 6, however, resulted in fairly good fits.

Even though the last model agrees with the experimetal data and other information available on the system, it need not be unique or final. In fact, the solution obtained was not unique and an additional constraint had to be imposed on the model. This means that a "simpler" model could probably be found to fit

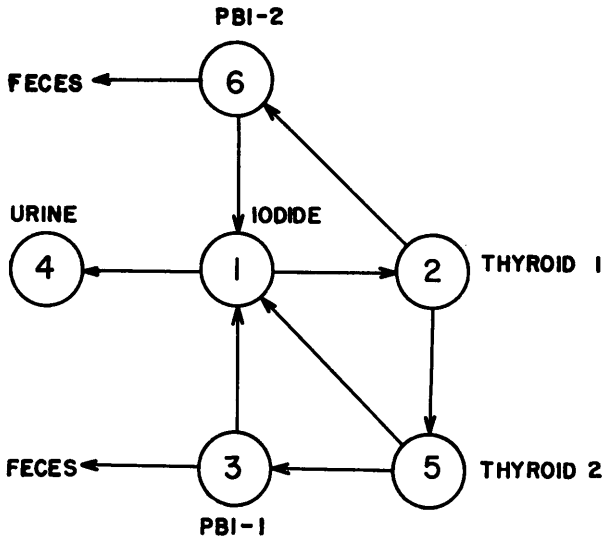


FIGURE 5

Iodide release path from thyroid compartment 5.

the data as well. Knowledge of the physiology of the system, however, suggested the configuration chosen.

The analysis of the thyroid system is still in progress and a number of studies have not yet been fitted to the model. In addition, studies [6] with labeled T_3 and

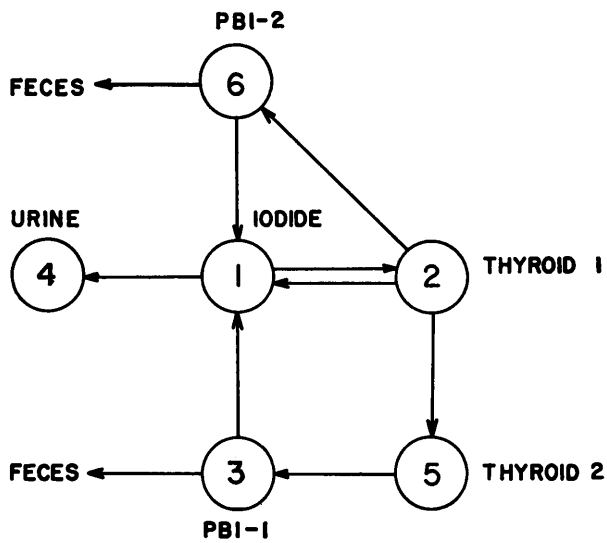


FIGURE 6

Iodide release path from thyroid compartment 2.

T_4 are also in progress. It is believed that these additional studies will permit further development of the model.

10. Conclusion

The combination of statistical procedures with analytical methods, aided by modern high-speed computers, has made possible a rational approach to the development of models for biological systems studied. The thyroid system was presented as an example to bring out the kind of problems encountered in radioactive tracer experiments. The actual models developed here should not be taken as final.

To permit the application of the various techniques described, certain empirical procedures had to be employed. It is hoped that some of the present theoretical inadequacies may be eliminated to permit a more systematic, rigorous, and efficient development of models.

A number of aspects of the problem have not been discussed because not much has been done on them as yet. These include the interpretation of the model in terms of the actual system, physiological significance for changes in the values of model parameters, and others.

Applications of the procedures discussed have been made to systems other than the thyroid, and useful models were derived for them. Although one cannot guarantee uniqueness for the models derived, the development of a model brought out very clearly inconsistencies in the originally assumed models.

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